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Ultrasound backscatter measurements of intact human proximal femurs—Relationships of ultrasound parameters with tissue structure and mineral density

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ABSTRACT

Ultrasound reflection and backscatter parameters are related to the mechanical and structural properties of bone *in vitro*. However, the potential of ultrasound reflection and backscatter measurements has not been tested with intact human proximal femurs *ex vivo*. We hypothesize that ultrasound backscatter can be measured from intact femurs and that the measured backscattered signal is associated with cadaver age, bone mineral density (BMD) and trabecular bone microstructure. In this study, human femoral bones of 16 male cadavers (47.0 ± 16.1 years, range: 21–77 years) were investigated using pulse-echo ultrasound measurements at the femoral neck in the antero-posterior direction and at the trochanter major in the anteroposterior and lateromedial directions. Recently introduced ultrasound backscatter parameters, independent of cortical thickness, *e.g.*, time slope of apparent integrated backscatter (TSAB) and mean of the backscatter difference technique (MBD) were obtained and compared with the structural properties of trabecular bone samples, extracted from the locations of ultrasound measurements. Moreover, more conventional backscatter parameters, *e.g.*, apparent integrated backscatter (AIB) and frequency slope of apparent integrated backscatter (FSAB) were analyzed. Bone mineral density of the intact femurs was evaluated using dual energy X-ray absorptiometry (DXA). AIB and MDB measured from the femoral neck correlated significantly ($p < 0.01$) with the neck BMD ($R^2 = 0.44$ and 0.45), cadaver age ($R^2 = 0.61$ and 0.41) and several structural parameters, *e.g.*, bone volume fraction ($R^2 = 0.33$ and 0.39, $p < 0.05$ and $p < 0.01$), respectively. To conclude, ultrasound backscatter parameters, measured from intact proximal femurs, are significantly related ($p < 0.05$) to structural properties and mineral density of trabecular bone.

Introduction

During the last decades the life expectancy in the western countries has increased, leading to an increase in the number of elderly. Evidently, this leads to a significant increase in the number of patients suffering from osteoporosis and related fractures. This will cause a significantly increased burden to the healthcare system. Currently, diagnostics of osteoporosis is based on areal bone mineral density determined using dual energy X-ray absorptiometry (DXA) [1]. This is problematic since many health centers do not have a DXA-scanner due to personnel, cost and space limitations. Thus, it has been estimated that as many as 75% of the osteoporotic patients lack diagnosis and medication [2]. Therefore, it is important to develop low cost portable tools for cost effective osteoporosis diagnostics in the future. Quantitative ultrasound (QUS) has provided diagnostically valuable information on bone mechanical, structural and compositional properties *in vitro* [3–10]. Furthermore, QUS (axial and through transmission) has shown potential in clinical assessment of fracture risk [11]. However, to predict fracture susceptibility at the most severe fracture sites, *e.g.*, proximal femur, development of techniques that allow local measurements at these sites is essential [12,13]. In order to rationalize the fracture risk prediction and fracture prevention, innovative combinations of cost effective tools are needed. A new screening tool, WHO fracture risk assessment tool (FRAX), has been introduced for fracture prediction at the basic healthcare [14]. In a recent study the FRAX and DXA based osteoporosis treatment proposals were efficiently predicted by combining information from pulse-echo (PE) ultrasound measurements and the FRAX tool [15]. Thus, FRAX and ultrasound provide promising possibilities

Abbreviations: AP, anteroposterior; AIB, apparent integrated backscatter; MBD, mean of backscatter difference spectrum; BMD, bone mineral density; DXA, dual energy X-ray absorptiometry; FSAB, frequency slope of apparent integrated backscatter; FRAX, fracture risk assessment tool; LM, lateromedial; PBS, phosphate buffered saline; PE, pulse-echo; QUS, quantitative ultrasound; TSAB, time slope of apparent integrated backscatter.

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for osteoporosis diagnostics and assessment of fracture risk at the basic healthcare.

Ultrasound transmission parameters, *i.e.*, broadband ultrasound attenuation [16–18] and speed of sound [16,17], and PE parameters, such as broadband ultrasound backscatter [6,16,17], integrated reflection coefficient (IRC) [16,17], apparent integrated backscatter (AIB) [6,7,19,20], time slope of apparent backscatter (TSAB) [7], frequency slope of apparent backscatter (FSAB) [7,19,20], mean of backscatter difference spectrum (MBD) [21] and slope of backscatter difference spectrum (SBD) [21] are related to bone composition, structure and mechanical properties. When estimating the mechanical strength of femoral neck, the cortical tissue plays a key role [22]. However, also the trabecular structure significantly contributes to bone strength [23]. For optimal prediction of hip fracture the measurements should be conducted at the proximal femur [12]. Unfortunately, there is no data available on PE ultrasound measurements of intact human proximal femurs *ex vivo*. We hypothesize that ultrasound backscatter can be measured from the intact proximal femur and the measured backscattered signal is related to bone mineral density and microstructure of the trabecular bone. Therefore we investigate the feasibility of PE measurements of intact proximal femurs *ex vivo* and evaluate the relationships between the backscatter parameters, trabecular bone microstructure, bone mineral density and cadaver age. Furthermore, we also investigate the feasibility of novel backscatter parameters that are independent of cortical thickness or soft tissue composition.

Materials and methods

Outline of the study

Sixteen male human proximal femurs were obtained from Kuopio University Hospital. The age range of the cadavers was 21–77 years (mean 47.0 ± SD 16.1 years). Ethical permission was granted by the National Authority of Medicolegal Affairs (TEO, 5783/04/044/07). None of the cadavers had any pre-existing conditions that might have affected bone metabolism. First, the intact proximal femurs were measured using both DXA and ultrasound. Subsequently, a band saw was used to remove the cortex from the trochanter major and to extract transversal cross section from the femoral neck. Thereafter, cylindrical trabecular samples (diameter 10 mm, length 10–15 mm) were extracted from these locations using a coring tool (Fig. 1a). The trabecular bone samples were imaged with high-resolution microcomputed tomography (microCT).

Dual-energy X-ray absorptiometry

After soft tissue removal the bone mineral density (BMD [g/cm^2]) of intact proximal femurs was determined with a DXA device (Lunar Prodigy, GE Healthcare Ltd., Madison, WI, USA), using the clinical hip measurement protocol. During the measurement, the proximal femurs were positioned according to the *in vivo* pelvic anatomy in a plastic container filled with 160 mm in height of phosphate buffered saline (PBS) to mimic the soft tissue. Neck, trochanter and total BMD were evaluated at the standard regions with the software provided by the manufacturer.

MicroCT measurements

Structural parameters of the trabecular bone were determined with a microCT (Skyscan-1172, Bruker microCT, Kontich, Belgium), using an isotropic voxel size of 14 μm, a tube voltage of 100 kV, a tube current of 100 μA, a 0.5 mm aluminium filter, and 10 repeated scans. The inner part of each sample was included in the structural analysis by choosing a cylindrical region of interest with a diameter of 8 mm. Further, 20 slices (= 0.28 mm) at the top and bottom of each sample were excluded from the analysis.

Standard parameters were calculated to quantify the bone microstructure. Trabecular bone volume fraction (BV/TV [%]), average trabecular thickness (Tb.Th [μm]), number (Tb.N [μm^{-1}]) and separation (Tb.Sp [μm]) were calculated. Moreover, the structural model index (SMI [−]) was determined, in which plate- and rod-like structures correspond to the values of zero and three, respectively. All analyses were performed using CTAn software (V. 1.13.2.1, Bruker microCT, Kontich, Belgium).

Pulse-echo ultrasound measurements

Pulse echo ultrasound measurements were conducted with an ultrasound system (UltraPAC, Physical Acoustic Co., Princeton, NJ, USA) consisting of a 500 MHz A/D board and a 0.2 to 100 MHz pulser-receiver board. A sampling frequency of 125 MHz and a 5 MHz point target focused transducer (V307, Panametrics Inc., Waltham, MA, USA) were used in the measurements. The focal distance and length (−6

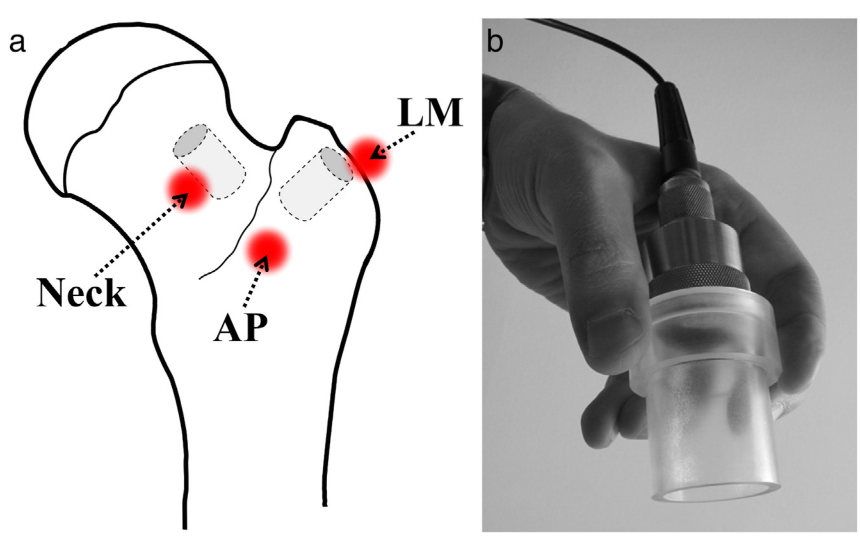

Fig. 1. (a) Trabecular samples taken from the femoral neck and trochanter are indicated with cylinders delineated with a dashed line. Locations and directions for ultrasound measurement at femoral neck (Neck), trochanter in lateromedial (LM) and anteroposterior (AP) are highlighted with red spots and dashed arrows, respectively. (b) Photograph of the ultrasound probe (focal distance of 50.9 mm) and a cylindrical holder by which the optimal focusing to the bone surface was realized. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).